

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> <b>A61K 9/00, 9/16</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 94/28871</b> <b>(43) International Publication Date:</b> 22 December 1994 (22.12.94)
<b>(21) International Application Number:</b> PCT/US94/06394 <b>(22) International Filing Date:</b> 7 June 1994 (07.06.94)  <b>(30) Priority Data:</b> 08/072,718                      7 June 1993 (07.06.93)                      US  <b>(71) Applicant:</b> ENDOCON, INC. [US/US]; 1 Foxhill Road, P.O. Box G, South Walpole, MA 02071 (US).  <b>(72) Inventor:</b> LEONARD, Robert, J.; 4 Hutchins Circle, Lynnfield, MA 01940 (US).  <b>(74) Agent:</b> GATES, Edward, R.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).		<b>(81) Designated States:</b> AU, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> IMPLANT STIMULATED CELLULAR IMMUNITY  <b>(57) Abstract</b>  Methods and products for stimulating cell-mediated immunity against intracellular pathogens are provided. A foreign body is implanted into a subject to induce the formation of a fibrous capsule defining a chronic, local inflammatory site. An antigen associated with a pathogen then is presented at that site at a level appropriate for stimulating cell-mediated immunity. The antigen is presented by a solid, immunogenic, subcutaneous implant containing the antigen.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

-1-

IMPLANT STIMULATED CELLULAR IMMUNITYField of the Invention

This invention relates to methods and products for stimulating in vivo cytotoxic T lymphocytes capable of killing cells infected with a pathogen.

Background of the Invention

Advances in medicine and public health have irradiated or significantly reduced the incidence of serious illness or death caused by many pathogens. Nevertheless, infectious diseases still are responsible for many serious health problems.

Technological advances in medicine, ironically, also have created some of the present, unsolved problems related to infectious disease. For example, immunosuppression resulting from medical treatments for cancer and transplants compromise the patient's defense system and create the opportunity for infection. Likewise, the use of antibiotics in hospitals has resulted in new strains of pathogens that are resistant to conventional antibiotic treatment.

One of the most serious health risks has resulted from a relatively new pathogen, the human immunodeficiency virus (HIV). This virus has had devastating effects, particularly in that it opens the door to infection by a variety of pathogens that previously were unimportant. Despite billions of dollars in research, an effective treatment for HIV infection has not been discovered to date.

The immune system is adapted among other things to protect mammals against infectious disease. It is comprised of cells derived from totipotent stem cells, and includes lymphocytes, monocytes/macrophages and granulocytes. The lymphocytes are composed principally of two major groups: the thymus associated T cells and the antibody producing B cells. In birds, these antibody producing cells clearly originate in the bursa, thus, B cells. It is not at all

-2-

clear that such a system exists in mammals. The term B cell, however, is utilized simply by convention. T cells represent the most complex subclass in view of the range of specialized functions assumed by distinct subgroups. Certain T cells act to regulate T and B cell function by the production of cytokines and by contact. Other T cells can terminally differentiate into cytotoxic T lymphocytes (CTLs), which are responsible for cell-mediated immunity by lysing pathogen-infected cells.

CTLs are of two lineages, characterized by the nature of the markers which they carry on their cell surface. These markers can be CD4 or CD8, the most predominant variety. The conditions required to cause a CD8 or CD4 cell to differentiate into a CTL are not well understood and are only now beginning to be elucidated. These conditions are complex and differ for CD8 and CD4 cells; for either cell type, various intercellular message molecules (cytokines) are involved. Cytokines play a major role in all forms of immunity and are at the heart of the inflammatory response. Since cytokines are so significant to the functional properties as well as specific differentiation of cell types --including whether or not a T cell expresses CD4 or CD8-- the use of cytokines as a therapeutic modality has been attempted, unfortunately with only limited success.

Other cells of the immune system include those cells which are of the monocyte/macrophage lineage as well as granulocytes such as neutrophils. Macrophages arise from specific monocyte stimulation and represent one of the most versatile cells of the immune system, able to lyse and/or engulf foreign bodies (bacteria, viruses, etc.) and scavenge toxins and tumor cells. Macrophages respond to and are the source of numerous cytokines, including cytokines that are responsible for T cell differentiation. Macrophages also present antigen to both CTLs and B cells in connection with stimulating cell-mediated immunity or inducing a humoral antibody response, respectively.

-3-

One approach to the treatment of infectious disease is the use of vaccines. These are preparations that contain immunogens that are modified so as to be incapable of producing the disease state, but capable of producing immunity against the pathogen. Vaccines have been very successful in treating some infections, but somewhat ineffective in treating others. This is perhaps due to the combination of the interaction between two complex systems, the host's immune system and the pathogen's system of replication.

For many years, various investigators have attempted to make improved vaccines for the purpose of enhancing the immune response or facilitating delivery of the vaccine. Some approaches have involved formulations adapted to provide sustained release of antigen. These approaches typically are adapted for stimulating a B cell response and are evaluated by measuring circulating antibody. They sometimes involve the introduction of agents which tend to suppress the local immune response to facilitate obtaining a desired level of circulating antigen. Presumably this is to ensure that antigen is delivered to regions of localized B cell differentiation. In one recent patent, U.S. patent no. 5,008,116, a different approach was taken; the implant was adapted to hold antigen at the site of the implant and to stimulate or suppress the immune response at that site. Antigens were physically entrapped in or chemically cross-linked to the interior of microporous microparticles. The micropores were of a size that would permit cellular infiltration. Agents for recruiting lymphocytes to the microparticles were suggested for inclusion in the microparticles.

One recent approach to treating a subject infected with the human immunodeficiency virus was to remove a sample of the subject's lymphocytes and stimulate them in vitro in the presence of an antigen associated with a specific pathogen. This produced a sub-sample of cells which was enriched for

-4-

cytotoxic T lymphocytes that recognized the antigen. The sub-sample of cytotoxic T lymphocytes was shown to be capable of lysing pathogen-infected cells of the subject. Accordingly, the sub-samples were administered to the subjects from which they were derived to treat the subject for the infectious disease.

It also was reported that CD8 T cells recognize antigenic peptides presented by MHC class I molecules and that for some infections, only a small number of peptides ("immuno-dominant peptides") are capable of initiating the terminal differentiation of a CD8 cell into a cytotoxic T lymphocyte. It further was reported that cytotoxic T lymphocytes specific for such immuno-dominant peptides are subject-specific and dominate the lytic response. Accordingly, when a subject's cytotoxic T lymphocytes were removed for in vitro enrichment as described above, a panel of peptides was used to ensure that the appropriate antigen was present for stimulating the subject-specific cytotoxic T lymphocyte response. In addition, it was suggested that such subject-specific peptides, once identified, could be administered intravenously together with the sub-sample of cytotoxic T lymphocytes to further stimulate a cytotoxic T lymphocyte response in the infected subject.

#### Summary of the Invention

The invention provides methods and products for stimulating in vivo cell-mediated immunity against pathogen-infected cells. It involves forming a local, chronic, inflammatory site and presenting an antigen associated with a pathogen at that site at a level appropriate for stimulating cell-mediated immunity against cells infected with the pathogen. Conditions are created at the local inflammatory site which favor the terminal differentiation of T cells into cytotoxic T lymphocytes. T

-5-

cells thus are continually recruited and expanded, thereby maximizing the potential for a vigorous cytotoxic response against the pathogen infected cells.

According to one aspect of the invention, novel sustained-released devices are provided. The devices are solid, immunogenic implants that induce, when implanted subcutaneously, the formation of a subcutaneous fibrous capsule defining a chronic inflammatory site. The implant contains an antigen associated with a pathogen. The implant is constructed and arranged to present the antigen for an extended period of time at a level appropriate for stimulating cell-mediated immunity, but at a level that does not induce tolerance.

The implant can be free of materials that suppress the immune response.

It is preferred that the implant contain a cytokine or a combination of cytokines for stimulating cell-mediated immunity. Most preferably the implant includes the cytokines interleukin 2, interleukin 4, interleukin 8, interleukin 12 and interferon  $\gamma$ , and it is preferred that the implant be constructed and arranged to deliver the cytokine for the same period of time that the antigen is being presented. The implant also can contain an agent for suppressing the conversion of macrophages into foam cells, and preferably the agent is an antioxidant.

According to another aspect of the invention, a subcutaneous fibrous capsule defining a chronic, local, inflammatory site is created in the subject by implanting subcutaneously a foreign body. An antigen associated with the pathogen then is presented continuously at a level appropriate for stimulating cell-mediated immunity, but at a level that does not induce tolerance. The antigen can be delivered using the devices described above.

The invention is particularly useful in the treatment of a subject infected with HIV. The chronic presentation of low doses of antigen in the environment of the fibrous capsule

-6-

can produce a cytotoxic T cell response directed to HIV infected cells. Such a cytotoxic T cell response is believed to be crucial in combating HIV infection and in preventing or arresting the development of Autoimmune Deficiency Syndrome (AIDS).

These and other aspects of the invention are described in greater detail below.

#### Detailed Description of the Invention

The invention involves forming a discrete, localized site of chronic inflammation, and using this site of inflammation as a local 'factory' for the production of cytotoxic T lymphocytes (CTLs) that will lyse cells infected with a specific pathogen. The expanded set of CTLs specific for a pathogen can eradicate or prevent the development of infection and also can be used to treat or arrest the development of cancers associated with infection.

When a solid, appropriately immunogenic, foreign body is introduced subcutaneously into a subject, a complex cellular response takes place which can result in the implanted object being encapsulated by a dense, fibrous capsule or compartment. The capsule is often characterized by new capillaries of both blood vessels and lymphatics. It is believed that this process begins with the chemotaxis of neutrophils and monocyte/macrophages to the site of "injury", probably enlisted by the presence of platelet derived growth factor (PDGF) and granulocyte/macrophage colony stimulating factor (GM-CSF). Macrophages apparently coat the object while simultaneously stimulating fibroblasts to begin constructing the fibrous "wall".

The capsule is highly structured, consisting of an inner lumen which can be lined with a layer of loose cellular material, a middle fibrous layer, and an outer loose connective tissue layer. The middle layer contains well-developed blood vessels and lymphatics and can be on the order, for example, of one half to thirty millimeters in



-7-

thickness (thousands of cells in thickness). The cellular compartment contains numerous round cells, macrophages and fibroblasts. The macrophages essentially coat the foreign body.

To achieve the complexity of the capsule and the apparent continuous cellular turnover, significant cytokine involvement is necessary, and it is believed that the lumen of the capsule is rich in cytokines.

Thus, a naturally-occurring, local inflammatory microenvironment is generated. The term "local inflammatory microenvironment" pertains to chronic, nonpathogenic, tissue responses characterized by: (1) macrophage stimulation and chemotaxis; (2) fibroblast stimulation and collagen deposition to form a dense fibrous capsule; and (3) development and infiltration of neovasculature (angiogenesis) and lymphatics. Not all foreign body responses and granulomas are the same as they relate to the relative presence of these components.

While the inventor does not wish to be bound by any theory of the invention, it is believed that chemotaxis attracts lymphocytes and macrophages to the site of inflammation within the vascularized fibrous capsule. Since the implant appears to remain coated with macrophages throughout, it is believed that the environment is particularly suited for antigen presentation to circulating lymphocytes and the creation of cytotoxic T lymphocytes.

It is unclear whether B cell stimulation is enhanced significantly. It may be that in certain circumstances, the B cell response is actually suppressed. If desired, the B cell response can be suppressed by including B cell suppressing agents in the implant.

As discussed above, the specific regulation of this inflammatory microenvironment appears to be modulated by naturally occurring, locally acting, cellular regulators, which affect colony stimulation, angiogenesis and tissue generation within the microenvironment. See, e.g., K. Arai

-8-

et al., "Cytokines: Coordinators of Immune and Inflammatory Responses", Annu. Rev. Biochem., 59:783-836 (1990). As discussed in parent application serial no. 07/699,763, this local microenvironment also can be manipulated by including cellular regulators in the implant. Such cellular regulators previously were used to affect the development of the fibrous capsule and its vasculature, thereby affecting the release-kinetics of drugs contained in the implant.

The present invention is designed to exploit this same local, inflammatory microenvironment for the purpose of generating a cell-mediated immune response to a pathogen-specific antigen. This local inflammatory microenvironment is particularly suited for recruiting the components of the cell-mediated immune system (e.g. macrophages, T-lymphocytes) and for creating an environment well-suited for the processing and presentation of antigens and the proliferation of CTLs.

The invention involves presenting an antigen associated with a pathogen within this microenvironment at a frequency or "dose" that is appropriate for stimulating cell-mediated immunity, but is low enough such that tolerance is not induced. As will be described in greater detail below, agents for inducing a vigorous CTL response may be introduced into this microenvironment (in addition to such agents as occur naturally within the local microenvironment).

The invention is useful in connection with stimulating cell-mediated immunity in mammals infected with an intracellular pathogen such as a virus, bacterium, fungus or protozoan. "Intracellular pathogen" means a disease-causing organism which resides, during at least part of its life cycle, within a host cell.

Viruses include but are not limited to those in the following families: picornaviridae; caliciviridae; togaviridae; flaviviridae; coronaviridae; rhabdoviridae; filoviridae; paramyxoviridae; orthomyxoviridae; bunyaviridae;

-9-

arenaviridae; reoviridae; retroviridae; hepadnaviridae; parvoviridae; papovaviridae; adenoviridae; herpesviridae; and poxyviridae.

Bacteria include but are not limited to: *P. aeruginosa*; *E. coli*; *klebsiella*; *serratia*; *pseudomonas*; *B. fragilis*; *P. cepacia*; *acinetobacter*; *N. gonorrhoeae*; *S. epidermis*; *E. faecalis*; *S. pneumoniae*; *S. aureus*; *haemophilus*; *neisseria*; *N. meningitidis*; *bacteroides*; *citrobacter*; *branhmella*; *salmonella*; *shigella*; *S. pyogenes*; *proteus*; *clostridium*; *erysipelotheix*; *lesteria*; *L. monocytogenes*; *pasteurella* *multocida*; *streptobacillus*; *spirillum*; *fusospirochetes*; *treponema pallidum*; *borrelia*; *actinomycetes*; *mycoplasma*; *chlamydia*; *rickettsia*; *spirochaeta*; *legionella pneumophila*; *mycobacteria*; *ureaplasma*; *streptomyces*; *C. difficile*; *trichomonas*; and *P. mirabilis*.

Fungi include but are not limited to: *cryptococcus neoformans*; *blastomyces dermatitidis*; *ajellomyces dermatitidis*; *histoplasma capsulatum*; *coccidioides immitis*; *candida*, including *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii* and *C. krusei*; *aspergillus*, including *A. fumigatus*, *A. flavus* and *A. niger*; *rhizopus*; *rhizomucor*; *cunninghamella*; *apophysomyces*, *saksenaea*, *mucor* and *absidia*; *sporothrix schenckii*; *P. brasiliensis*; *pseudallescheria boydii*; *T. glabrata*; and dermatophytes.

Treatment or prevention of infection by the following pathogens is preferred:

Human immunodeficiency virus (including, without limitation, HIV-1 and HIV-2); human T cell leukemia virus (including, without limitation, HTLV-I and HTLV-II); herpes virus (including, without limitation, Herpes simplex type 1 and type 2, Herpes zoster, and cytomegalovirus as well as epstein-barr virus; papillomavirus; hepatitis (including, without limitation hepatitis A, B and C); creutzfeldt-jacob virus; feline leukemia virus; micobacteria (including, without limitation, *M. tuberculosis* and *M. leprae*); pneumocystis carinii; *cryptococcus neoformans*; *candida*

-10-

(including, without limitation, candida albicans and candida tropicalis); mycoplasma; toxoplasma gondii, giardia lamblia; trypanosoma cruzi; organisms of the genus leishmani; and organisms of the genus plasmodium.

The invention is particularly useful when a T cell response is important in treating or preventing an infection by a pathogen. Most preferred is treatment or prevention of infection by human immunodeficiency virus.

The antigen selected for use in connection with the present invention will depend upon the particular disease being treated. It may be any antigen associated with the pathogen. The antigen can be a plurality of antigens or a single, defined epitope. It can be whole and/or attenuated virus, bacterium, fungus or protozoan. It can be any portion thereof, isolated according to procedures well known to those of ordinary skill in the art. For example, it can be a component of a pathogen isolated by disrupting the pathogen and isolating the component. It also can be produced by mutation and/or recombinantly as a mutant pathogen. It can be whole protein, fusion protein, peptide, or part of a vector such as part of a vaccinia vector. It further can be produced synthetically, such as a peptide synthesized de novo, or it can be derived from a related organism (e.g. Bacillus Calmette-Guerin (BCG) in the case of tuberculosis). The invention in its broadest aspect is not intended to be limited by the selection of the particular antigen or by its mode of preparation. Those of ordinary skill in the art will have available to them well characterized antigens previously used, for example, as vaccines for treating the various conditions discussed herein. Thus, as used herein, an "antigen associated with a pathogen" is any antigen that is capable of inducing a cell-mediated immune response and capable of being recognized by a cytotoxic T cell as part of the pathogen or a cell infected with the pathogen.

-11-

In the case of human immunodeficiency virus, many antigenic components have been well characterized and cloned. Some antigenic components include nuclear materials which are expressed on the surface of cells infected by the human immunodeficiency virus. Other antigenic components include peptides. The following is a list of epitopes reported to be capable of stimulating CTLs.

TABLE I  
CTL EPITOPES  
CLASS I RESTRICTED

<u>Protein</u>	<u>Amino Acids</u>	<u>Residues</u>	<u>HLA Type</u>	<u>Reference</u>
<u>nef</u>				
	66-80	VGEPVTOVPLRPMT	A1	1
	73-82	QVPLRPMTYK	A3.1	2
	73-82	QVPLRPMTYK	A3,A11,B35	3
	83-94	AAVDLSHFLKEK	A11	3
	93-106	EKGGEGLIHSQRR	A1	1
	113-128	WIYHTOGYFPDWQNYT	A1	1
	115-125	YHTQGYFPDWQ	B17	3, 4
	117-128	TQGYFPDWQNYT	B17,B37	3
	126-138	NYTPGPGVRYPLT	B7	3
	132-147	GVRYPITFGWCYKLVP	B18	3
	132-147	GVRYPITFGWCYKLVP	A1	1
	182-198	EWRFDSRLAFHHVAREL	A1	1
	192-206	HHVARELHPEYFKNC	A1	1
<u>pol</u>				
	172-196	IETVPVKLKPGMDGPKVKQWPLTEE	B8	5
	205-219	CTEMEKEGKISKIGP		?
6				
	325-349	AIFQSSMTKTLEPFRKQNPDIYIYQ	A11	5
	342-366	NPDIYIYQYMDLDYVGSDEIGQHR	A11	5
	359-383	DLEIGQHRTDIEELRQHLLRWGLTT	Bw60	5
	461-485	PLTEEALELELAENREILKEPVHGVY	A2	5
	495-519	EIQKQGQGWYQIYQEPFKNLKTG	A11	5
<u>gag</u>				
<u>p17</u> (MA)	18-42	KIRLRPGGKKKYKLKHIVWASRELE	Bw62	7
	21-35	LRPGGKKKYKLKHIV	B8	8
	69-93	QTGSEELRSLYNTVATLYCVHQRIE	A2	7
	71-85	GSEELRSLYNTVATL	A2	8
	86-115	YCVHQRIEIKEEQNKSKKKA	A2	9
<u>p24</u> (CA)	143-164	VHQAI SPRTLNAWVKVVEEKAF	Bw57	7
	143-157	VHQAI SPRTLNAWVK	?	8
	153-174	NAWVKVVEEKAFSPEVIMFSA	Bw57	7
	185-199	DLNTMLNTVGGHQAA	B14	8

-12-

	193-203	GHQAAMQMLKE	A2	10
	219-233	HAGPIAPGQMREPRG	A2	10
	253-267	NPPIPVGEIYKRWII	B8	11
	253-274	NPPIPVGEIYKRWIIILGLNKIV	B8	7
	262-284	YKRWIIILGLNKIVRMYSPTSILD	Bw62	7
	263-277	KRWIIILGLNKIVRMV	B27	12
	293-307	FRDYVDRFYKTLRAE	?	8
	303-314	TLRAEQASQEVK	B14	13
	305-313	RAEQASQEV	B14	7
	323-337	VQNANPDCKYILKAL	B8	8
	342-357	LEEMMTACQGVGGPG	?	8
	353-363	VGGPGHKARVL	?	8
p7 (NC)	418-433	KEGHOMKDCTERQANF	A2	10
	446-460	GNFLQSRPEPTAPPF	A2	10

env

gp120	34-55	LWVTVYYGVPVWKEATTTLFCA	A2	14
	105-117	HEDIISLWDQSLK	A2	15
	188-207	TTSYTLTSCNTSVITQACPK	A2	14
	219-307	SVEINCTRPNNNTRKSI	A2	14
	308-322	RIQRGPGRFVITIGK (mouse)	Dd	16
	308-322	RIQRGPGRFVITIGK	A2	15
	369-375	PEIVTHS	A2	14
	379-390	GGEFFYCNSTQL	A2	17
	416-435	LPCRIKQFINMWQEVGKAMY	A2	14
	421-439	KQIINMWQKVGKAMYAPPI	A2	15
	489-508	VKIEPLGVAPTKAKRRVVQR	A2	14
gp41	584-592	ERYLKDQQL	B14	13
	586-593	YLKDQQLL	B8	13
	770-780	RLRDLILLIVTR	A3.1	18
	827-841	DRVIEVVQGACRAIR	A2	15

CLASS II RESTRICTEDenv

gp120	309-316	IQRGPGRA	not
identified	19		
	310-316	QRGPGR(FVTI)	19
	312-318	GPGRAFV(TI)	19
	410-429	GSDTITLPCRIKQFINMWQE	DR4
gp41	579-590	RILVERYLKDQ	DPw4.2

It is to be noted that peptides may be subject specific. The subject then may need to be tested in advance of treatment according to the present invention to determine the identity of peptides most useful for stimulating cellular immunity. Such a procedure has been developed for subjects infected with human immunodeficiency virus, and is reported in PCT application PCT/US91/06441, publication no. WO92/04462, published March 19, 1992.

-13-

For influenza, a peptide fragment of influenza matrix protein shown to be restricted by HLA-A2 may be used: TKGILGFUFTLTV. This peptide, presented by self-MHC, has been shown to induce an increase in CTL proliferation in vitro in the presence of a combination of interleukin-12 and low-dose interleukin-2 (see M. Bertagnolli, B-Y Lin, D. Young and S. Herrmann, IL-12 Augments Antigen-Dependent Proliferation of Activated T-lymphocytes, J. Immun., V. 149, 3778-3783, No. 12, 12/15/92).

Reference also may be made to Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Co., Easton, PA 18042 (1990) for details with respect to the preparation of and commercial sources for various antigens and vaccines.

Cytokines may be delivered to the microenvironment and used to induce CTL proliferation. These introduced cytokines will augment the stimulatory effect of the cytokines occurring naturally in the microenvironment of the fibrous capsule. Cytokines are factors that support the growth and maturation of cells, including lymphocytes. Important to the invention herein is activating those cells responsible for cell-mediated immunity and the generation of CTLs. Cytokines are known to participate in such activation.

The following is a list of cytokines known to the inventor.

TABLE II

CYTOKINE	SOURCE	FUNCTION
<u>Interleukins</u>		
IL-1	Macrophage	Proliferation of activated T-cells; induction of macrophages to make cytokines
IL-2	T-cell	Activates killer cells
IL-3	T-cell	Growth, hematopoiesis
IL-4	T-cell	Growth, activation of T cells
IL-6	Macrophages	Growth of eosinophils

-14-

IL-7	Stromal cells	Growth of T cells
IL-8	Monocytes	Chemotaxis of T cells
IL-9		
IL-10		
IL-11		
IL-12		Activates T cells

#### Colony Stimulating Factors

GM-CSF	T-cell, macrophage	
Macrophage growth		
G-CSF	Fibroblasts	Growth of granulocytes
M-CSF	Fibroblasts	Growth of macrophage colonies

#### Tumor Necrosis Factors

TNF-alpha	Macrophages	Tumor cytotoxicity
TNF-beta	T-cells	Activates cytokine production

#### Interferons

IFN-gamma	T-cells	Activates macrophages
-----------	---------	-----------------------

#### Growth Factors

Platelet-derived growth factor	Platelets	
Epidermal growth factors		
Transforming growth factor		Macrophages
Fibroblast growth factor		

To promote the production of CTLs specific for antigen, use of the following combination of cytokines is preferred: IL-2, IL-4 and IL-12 for activating T cells; IL-8 for chemotactic recruitment of T cells; and interferon  $\gamma$  for activating macrophages.

The precise amounts of the foregoing compounds used in the implants of the invention will depend upon a variety of factors, including the nature of the antigen and the duration that the implant is to last. It will be understood, however, that daily presentation of very small amounts (picograms) are



-15-

required because only local presentation within the fibrous capsule are necessary to achieve the purposes of the invention.

The precise amounts can be determined without undue experimentation. The threshold amounts of cytokines to achieve a particular result have been tested in the art both in vivo and in vitro. The maximum amounts may be determined for achieving the highest levels of antigen-specific CTLs by formulating implants and testing for the presence of CTLs. It will be understood, however, that practically any amount, no matter how small, of the preferred cytokines should be useful in promoting the differentiation of precursor T cells into CTLs. It further will be understood that the art teaches that low doses of IL-2 with IL-12 are particularly useful in stimulating the production of CTLs (see M. Bertagnolli, id). It is believed that cytokines present in the following ranges will be quite suitable for achieving the purposes of the invention.

IL-2      10 picograms to 10 nanograms presented daily.

IL-12    10 picograms to 10 nanograms presented daily.

IFN  $\gamma$    10 picograms to 10 nanograms presented daily.

The antigens are presented subcutaneously as solid aggregates. A "solid aggregate" as used herein means an article of the type that can continue to present at its surface, over an extended period of time, an agent when implanted in vivo. Such devices can be constructed entirely of the agent itself, such as a totally melted and recrystallized pellet of antigen. They also can be constructed of the agent together with a bioerodible excipient such as cholesterol. They further can be contained in a nonerodible matrix such as a silastic matrix. Important for the purposes herein is that the device is capable of presenting the agents, i.e. antigens, at levels that are appropriate for stimulating cell-mediated immunity but at a dose that typically does not induce tolerance.

-16-

The solid aggregate devices useful herein thus can present continuously very small amounts of antigen for periods from two months to two years or more. The level of antigen present at the surface of the solid aggregate and taken up by the macrophages per day preferably is less than fifty micrograms per day, in order to prevent tolerance and most preferably is in the nanogram or even picogram range. Extremely low levels of presentation are possible because the conversion of T cells into CTLs occurs in the local microenvironment of the fibrous capsule.

Because the implant is coated entirely with macrophages in the lumen of the capsule, antigen is said to be presented, not released. Free antigen in the blood stream may not even be detectable, since macrophages preferably are binding all antigen within the capsule. Thus, the capsule is intended to create an environment rich in cells, cytokines and antigen for stimulating within the capsule the desired immune response. This differs from the prior art which does not teach implants containing antigen that form such capsules and which seek principally to produce a humoral response, and/or action at a distance.

It will be understood that IL-2 tends to prevent the induction of anergy in T cells. Likewise, CD28 stimulation by CD28 agonists including by anti-CD28 antibodies or fragments thereof tend to prevent the induction of anergy. Thus, higher doses of antigen may be presented in the presence of such substances without resulting in tolerance.

In preparing the implant, it is desirable to take into account the type of antigen being employed. It is believed that all cells expressing CD8 have the potential of developing into CTLs. Some, but not all, cells expressing CD4 have the same potential. CD8 cells have been reported to respond to peptides, not proteins. Therefore, if the antigen is only whole protein, it is possible that only CD4 cells will be stimulated. On the other hand, it has been reported that CD8 cells will respond only to particular

-17-

subject-specific, immunodominant peptides (See PCT publication no. WO92/04462), and therefore it may be desirable to prescreen a subject to identify those peptides to which the subject will respond maximally if using only peptides as an antigen. Nevertheless, the environment of the fibrous capsule may be such as to degrade protein, and CD8 cells still may be activated even when whole protein is delivered via the implant. It also will be understood by those of ordinary skill in the art that CD8 cells and CD4 cells respond to different cytokines and signals. Therefore, the antigen selected may also determine the accessory molecules presented with the antigen, such as cytokines or other signalling molecules.

As discussed above, INF  $\gamma$  may be included in the implant to activate macrophages for presenting antigen. Other materials also may be added to enhance the environment relative to macrophage or T cell activation. For example, oxidated LDL can stimulate macrophages to become foam cells. As discussed herein, the fibrous capsule defines a chamber that contains the implant. Surrounding the implant and within the chamber are a variety of cells, including both macrophages and foam cells. Macrophages are the precursors of foam cells and are activated to become foam cells by a variety of mechanisms. The most common mechanism is the interaction of a receptor on the macrophage with oxidated low-density lipoprotein (oxLDL). The oxLDL is produced by oxidizing low-density lipoprotein, and it has been reported that such oxidation is associated with sites of chronic inflammation. In some instances, it may be desirable to prevent such activation to maintain the macrophages in a state that is optimal for antigen presentation to T cells. IL-2 is believed to be one such agent. Activation also can be reduced by preventing the formation of oxLDL in the environment of the fibrous capsule. This can be achieved by continuously releasing an anti-oxidant into the environment of the inflammatory site. Anti-oxidants include vitamin E, butylated hydroxytoluene, etc.

-18-

As will be understood by those of ordinary skill in the art, virtually any molecule that participates in the conversion of a T cell to a CTL may be presented at the site of inflammation in accordance with this invention. Many such molecules are known to those of skill in the art, including, without limitation, anti-CD3 antibody and in particular solid phase anti-CD3 antibody, phorbol esters, aloe antigen, B7 and the like. The implants of the invention also may include adjuvants for enhancing the CTL response.

The implants of the invention may be used prophylactically or therapeutically. When used prophylactically, they typically are given to a subject that is at risk of being infected by a pathogen. When used therapeutically, they typically are given to a subject that is known to have or that is suspected of having developed an infection by a pathogen. As used herein, a subject means a human, primate, horse, cow, sheep, goat, pig, dog, cat or rodent. The preferred sustained release devices are solid, immunogenic, subcutaneous implants that induce the formation of a fibrous capsule defining a chronic inflammatory site. By "solid" implant it is meant solid enough to permit the formation of a dense fibrous capsule surrounding the implant prior to substantial dissipation or degradation of the implant.

Suitable implants for use in connection with the invention are compressed pellets wherein an antigen, alone or together with an excipient, is compressed under high pressure into a solid implant. A preferred implant is a nonpolymeric system comprising a fused pellet. In a fused pellet, an antigen, a carrier, or both are melted and recrystallized to form a crystalline matrix of the antigen and/or nonpolymeric carrier. In a totally fused pellet, both the antigen and carrier are melted and recrystallized. In a partially fused pellet, only the carrier is melted and recrystallized, thereby capturing the antigen in the crystalline matrix of the carrier. A partially fused pellet is particularly suitable

-19-

for the delivery of proteins or peptides whose conformation and properties might be compromised if heated to their melting temperature. Thus, a carrier having a lower melting temperature than the peptide or protein is heated to the melting point of the carrier, and then recrystallized to form a matrix encapsulating the peptide or protein. Stearols are particularly suited for melting and recrystallization. For example, various cholesterol-type compounds including cholesterol acetate may be used. Compounds such as palmitic acid also may be used. The particular methods for forming fused pellets are disclosed in U.S. patent nos. 4,748,024, 4,892,734 and 5,039,660 (all to Leonard), the entire disclosures of which are incorporated herein by reference.

Another preferred embodiment is the use of a jacketed implant which is the subject of co-pending application serial no. 07/565,273, "Multiple Drug Delivery System", the disclosure of which is incorporated herein by reference. In this embodiment, cellular regulators are combined in a matrix with a bioerodible polymer, such as poly-DL lactide or glycolyde, as a sheath around a core implant including the antigen. The sheath is formed so as to gradually erode, releasing the cellular regulator and preparing the tissue environment for presentation of the antigen contained in the core. These regulators would be released early and for the time period of formation of the fibrous capsule. Whether or not the sheath contains a cellular regulator, the sheath or a complete coating can be used to delay the presentation of antigen until a dense fibrous capsule has been formed.

The antigen also may be presented in polymeric systems. A polymeric system consists of matrices of polymers combined with the antigen. Such systems include systems in which the antigen is distributed in or covalently attached to a biodegradable polymer matrix. Biodegradable polymers that have been used in such systems include hydroxycarboxylic acids, especially lactic acid and glycolic acid. Cholesterol and ethylene vinyl acetate copolymers have also been used.

-20-

See, for example, U.S. Patent No. 4,591,496 issued to Cohen et al., which describes a polymeric system consisting of mixing a drug and a polymer, e.g. ethylene-vinyl acetate copolymer powders, below the glass transition temperature of the polymer, and compressing the mixture at a temperature above the transition point.

Bioerodible polymers include hydroxycarboxylic acids, especially lactic acid and glycolic acid, polycaprolactone and copolymers thereof. For instance, various proportions of lactide and glycolyde can be employed, such as 50/50, 65/35, 75/25, and 85/15 percent weight ratios of poly (DL-lactide-co-glycolide). In addition, substantially 100% weight percent poly (DL-lactide), poly (L-lactide), and polyglycolide can be used. Bioerodible polymers of this type are obtained from Birmingham Polymers, Inc., Birmingham, Alabama 35222. Copolymers of gluconic acid and ethyl-L-glutamic acid and other polypeptides can be used, as well as poly(orthoesters) (Choi et al., U.S. Patent No. 4,093,709) and poly(orthocarbonate) (Schmitt, U.S. Patent No. 4,346,709). Also, poly(acrylate) materials can be employed, such as copolymers of acrylic and methacrylic acid esters or copolymers of methacrylic acid and methyl methacrylate (Sothmann et al., U.S. Patent No. 4,351,825). Cholesterol and ethylene vinyl acetate copolymers can also be used. See for example, U.S. Patents 4,452,775 (J.S. Kent) and 4,591,496 (J.M. Cohen et al.), respectively.

If a polymeric matrix is employed, it must be fashioned to be a "solid aggregate" as defined herein. The matrices of the prior art typically were used for releasing circulating amounts of antigen for inducing a B-cell response. They also typically were porous, permitting tissue infiltration and/or were erodible or nonimmunogenic to an extent where a dense fibrous capsule did not form. Knowing as a result of this invention that a dense fibrous capsule can form as a result of implanting a solid, immunogenic foreign body and that such

-21-

a capsule is desirable for presenting antigen, those of ordinary skill in that art will be able to manufacture solid, immunogenic implants from polymer matrices.

Other implants are made of inert materials. The term "inert" refers to implants that are not eroded or otherwise structurally compromised when implanted. Exemplary materials include polytetrafluoroethylene (Teflon®), plastic, silicone or ceramic materials.

The invention also provides methods and products for treating cancer. Certain viruses are associated with cancer and are expressed on neoplastic cells, but not normal cells. These include hepatitis B, Epstein Barr virus and human papilloma virus. The methods and products of the invention thus are adapted for stimulating cell-mediated immunity against viral cancer antigens for the purpose of producing cytotoxic T cells against neoplastic cells. These viral antigens are known to those of ordinary skill in the art. The best characterized cancers include melanomas, ovarian cancers and renal cancers.

#### Example I

A solid implant for stimulating cell-mediated immunity against influenza virus.

The peptide fragment of influenza matrix protein, described above, is homogeneously distributed and captured in a matrix of a melted and recrystallized nonpolymer carrier. The carrier, powdered cholesterol acetate obtained from Sigma Chemical Co. of St. Louis, MO., has a lower melting point than the influenza peptide and is selected so that when a homogeneous mixture of the cholesterol acetate and peptide is heated, a partial melt may be formed with substantially all of the carrier melting and substantially all of the peptide not melting. The partial melt then is allowed to cool, with the cholesterol acetate recrystallizing to form the hardened pellet and capture the influenza peptide.

-22-

Pellets of the foregoing type are formed as described in U.S. patent 5,039,660, the entire disclosure of which is incorporated herein by reference. Briefly, fluorocarbon tubes that are straight cylinders having an inner diameter of 0.095 inches, an outer diameter of 0.135 inches and a length of 0.625 inches are provided. Such tubing is sold under the name of Teflon-TFE, Norton Chemplast, Inc., of Wayne, N.J. Next, the powdered cholesterol acetate and the influenza peptide are combined in approximate proportions of 10% peptide and 90% cholesterol acetate (weight/weight). The mixture is dispensed into the fluorocarbon tube in an amount such that when dried, the remaining powder equals 2ml in depth after a first compression phase. Approximately 20g of powder in the form of a paste is added to the tube using a pipette. A vacuum force then is applied across the filter sheet to evaporate the solvent.

Accurate dispensing of the starting materials can be achieved by forming the paste which is made from a powdered mixture of the starting materials and a liquid such as ethyl alcohol. The alcohol may be dried out of the starting materials after dispensing, utilizing, for example, a standard vacuum oven. By forming a paste, the material can be dispensed accurately from a standard, automated device such as a micropipeter. The paste may be manufactured to have the flow characteristics of ordinary toothpaste. For further details, see U.S. patent 4,892,734, issued 1/9/90 to Leonard, the entire disclosure of which is incorporated herein by reference.

The tube is plugged with a 3mm piece of Teflon beading, and the dry mixture then is compressed against the plug, using a steel pin inserted into the end of the tube opposite the plug. The plug end is held against a solid surface to prevent the plug from being expelled from the tube. The degree of compression corresponds to about 2200 p.s.i. for three seconds. The tube containing the compressed dry mixture then is transferred on the pin to an oven preheated



-23-

to 180°F. The tube is exposed to this environment for 30 seconds. The tube then is removed from the oven and is compressed by hand at about 150 p.s.i. for about six seconds as the partially-melted material cools and recrystallizes to form a hardened pellet. The mixture is allowed to cool for an additional three minutes without any compression and, finally, the plugs and pellets are ejected from the tubes. The pellets then may be used for subcutaneous implantation. Pellets of the foregoing type are commercially available from Endocon, Inc., Walpole, MA, U.S.A.

One method of administering the implant is using an injector of the type described in U.S. patent 4,846,793, the entire disclosure of which is incorporated herein by reference. Such injectors also are sold by Endocon, Inc.

The formed pellet is implanted subcutaneously in a mammal and a fibrous capsule surrounding the implant is permitted to form. Each week, a sample of peripheral blood is collected and tested for the presence of cytotoxic T cells active against cells infected with influenza virus. Procedures for collecting blood and testing for the presence for influenza-specific cytotoxic T cells are well known to those of ordinary skill in the art.

#### Example II

A solid implant for stimulating cell-mediated immunity against influenza virus, the implant containing cytokines.

Same as Example I, except that the mixture includes 10% influenza matrix peptide, 88% cholesterol acetate, 0.8% Interleukin-12, 0.4% Interleukin-2, 0.4% Interleukin-8, 0.2% Interleukin-2 and 0.2% Interferon  $\gamma$ .

#### Example III

A solid implant for stimulating cell-mediated immunity against human immunodeficiency virus.

The procedure of Example I is followed, except that 90% cholesterol acetate and 10% of an HIV-CTL epitope are used as starting materials.

-24-

Example IV

A solid implant for stimulating cell-mediated immunity against human immunodeficiency virus, the implant containing subject-specific immunodominant peptides.

The same as Example I, except that 95% cholesterol acetate and 5% of a subject-specific immunodominant peptide are used as starting materials. The subject-specific immunodominant peptide is identified as described in U.S. patent application serial no. 07/578,828, filed September 6, 1990, the entire disclosure of which is incorporated herein by reference.

Example V

The same as Example I, except that 80% cholesterol acetate, 15% influenza peptide, 4% Interleukin-12 and 1% Interleukin-2 are used as starting materials.

Example VI

The same as Example I, except that 96% cholesterol acetate, 2% influenza peptide, 1% Interleukin-12, 0.5% Interleukin-2 and 0.5% interferon  $\gamma$  are used as starting materials.

EQUIVALENTS

Those skilled in the art will be able to ascertain, using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein.

These and all other equivalents are intended to be encompassed by the following claims.

REFERENCES

- (1) B. Autran, et al, as reported in reference 8.
- (2) Koenig S, Fuerst TR, Wood LV, Woods RM, Suzich JA, Jones GM, de la Cruz VF, Davey RT, Jr, Venkatesan S, Moss B, Biddison WE, and Fauci AS: Mapping the fine specificity of a cytolytic T cell response to HIV-1 nef protein. J. Immunol 1990; 145: 127-135.

-25-

- (3) Culman B, Comard E, Kieny M-P, Guy B, Dreyfus F Saimot A-G, Sereni D, and Levy J-P: Six epitopes reactant with human cytotoxic CD8+ T cells in the central region of the HIV-1 nef protein. *J Immunol* 1991; 146: 1560-1565.
- (4) Culmann B, Comard E, Kieny M-P, Guy B, Dreyfus F Saimot A-G, Sereni D, and Levy J-P: An antigen peptide of the HIV-1 nef protein recognized by cytotoxic T lymphocytes of seropositive individuals in association with different HLA-B molecules. *Eur J Immunol* 1989; 19: 2883-2386.
- (5) Walker BD, Flexner C, Birch-Limberger K, Fisher L, Paradis TJ, Aldovini A, Young R, Moss B, and Schooley RT: Long-term culture and fine specificity of human cytotoxic T-lymphocyte clones reactive with human immunodeficiency virus type 1. *Proc Natl Acad Sci (USA)* 1989; 86: 9514-9518.
- (6) Hosmelin A, Clerici M, Houghten R, Pendleton CD, Flexner C, Lucey DR, Moss B, Germain RN, Shearer GM, and Berrofsky JA. An epitope in human immunodeficiency virus 1 reverse transcriptase recognized by both mouse and human cytotoxic T lymphocytes. *Proc Natl Acad Sci (USA)* 1990; 87: 2344-2348.
- (7) Johnson RP, Trocha A, Yang L, Mazzara GP, Panicali DL, Buchanan TM, and Walker BD: HIV-1 gag-specific CTL recognize multiple highly conserved epitopes: Fine specificity of the gag-specific response defined using unstimulated PBMC and cloned effector cells. *J Immunol* 1991; 147: 1512-1521.
- (8) Nixon D, and McMichael AJ: Cytotoxic T-cell recognition of HIV proteins and peptides. *AIDS* 1991; 5: 1049-1059.

-26-

(9) Achour A, Picard O, Zagury D, Sarin PS, Gallo RC, Naylor PH, and Goldstein AL: HGP-30, a synthetic analogue of human immunodeficiency virus (HIV) p17, is a target for cytotoxic lymphocytes in HIV-infected individuals. Proc Natl Acad Sci (USA) 1990; 87: 7045-7049.

(10) Claverie J-M, Kourilsky P, Langlade-Demoyen P, Chalufour-Prochnicks A, Dadaglio G, Tekaia F, Plata F, and Bougueleret K: T-immunogenic peptides are constituted of rare sequence patterns. Use in the identification of T epitopes in the human immunodeficiency virus gag protein. Eur J Immunol 1988; 18: 1547-1553.

(11) Gotch FM, Nixon DF, Alp N, McMichael AJ, and Boysiewicz LK: High frequency of memory and effector gag specific cytotoxic T lymphocytes in HIV seropositive individuals. Internat Immunol 1990; 2: 707-712.

(12) Nixon DF, Townsend ARM, Elvin JG, Rizza CR, Gallway J. and McMichael AJ: HIV-1 gag-specific cytotoxic T lymphocytes defined with recombinant vaccinia virus and synthetic peptides. Nature 1988; 336: 484-487.

(13) Johnson RP, Troch A, Earl P, Moss B, Buchanan TM, and Walker BD: Identification of a conserved CTL epitope in the HIV-1 gp41 envelope recognized by B8- and B14-restricted T-lymphocyte clones. VII International conference on AIDS. Florence, June 1991 [abstract WA.1206].

(14) G. Dadaglio, et al, as reported in reference 8.

(15) Clerci M, Lucey DR, Zajac RA, Boswell RN, Gabel HM, Takasahi H, Berzofsky JA, and Shearer GM. Detection of cytotoxic T lymphocytes specific for synthetic peptides of gp160 in HIV-seropositive individuals. J. Immunol 1991; 146: 2214-2219.

-27-

(16) Takahashi H, Cohen J, HOSmalin A, Cease KB, Houghten R, Cornette JL, DeLisi C, Moss B, Germain RN, and Berzofsky JA: An immunodominant epitope of the human immunodeficiency virus envelope glycoprotein gp160 recognized by class I major histocompatibility complex molecule-restricted murine cytotoxic T lymphocytes. Proc Natl Acad Sci (USA) 1988; 85: 3105-3109.

(17) Walker BD, and Plata F: Cytotoxic T lymphocytes against HIV. AIDS 1990; 4: 177-184.

(18) Takahashi K, Dai L, Fuerst TR, Biddison WE, Earl P, Moss B, and Ennis FA: Definition of a conserved HLA-A3, restricted CTL epitope on HIV-1 gp41. VII International conference on AIDS. Florence, June 1991 [abstract WA, 1206].

(19) Weinhold KJ, Medrick HL, Place CA, and Sebastian NW: Cell-mediated cytolytic reactivities against epitopes contained within the V3 region of HIV-1 gp120. J Cell Biochem 1990; Supplement 14D; pg 180; abs. L 550.

(20) Siliciano RF, Lawton T, Knall C, Karr RW, Berman P, Tregory T. and REingerz EL: Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: Effect of HIV sequence variation and a mechanism for CD4+ cell depletion. Cell 1988; 54: 561-575.

(21) Hammond SA, Obah E, Stanhope P, Monell CR, Strand M, Robbins FM, Bias WB, Karr RW, Koenig S, and Siliciano RF: Characterization of a conserved T cell epitope in HIV-1 gp41 recognized by vaccine induced human cytolytic T cells. J Immunol 1991; 146: 1470-1477.

I claim:

-28-

CLAIMS

1. A sustained-release device comprising,  
a solid, immunogenic, implant that induces when  
implanted subcutaneously the formation of a subcutaneous,  
fibrous capsule defining a chronic, inflammatory site, the  
implant containing an antigen associated with a pathogen and  
the implant constructed and arranged to present the antigen  
for at least two months at a level appropriate for  
stimulating cell-mediated immunity but at a level that does  
not induce tolerance.
2. A sustained-release device as claimed in claim 1 wherein  
the implant is free of materials that suppress the immune  
response.
3. A sustained-release device as claimed in claim 1 wherein  
the implant contains an agent for suppressing the conversion  
of macrophages into foam cells, the implant being constructed  
and arranged to deliver the agent for at least two months.
4. A sustained-release device as claimed in claim 1 wherein  
the implant contains an antioxidant for suppressing the  
conversion of macrophages into foam cells, the implant being  
constructed and arranged to deliver the antioxidant for at  
least two months.
5. A sustained-release device as claimed in claim 1 wherein  
the implant contains a cytokine for stimulating cell-mediated  
immunity, the implant being constructed and arranged to  
deliver the cytokine for at least two months.
6. A sustained-release device as claimed in claim 5 wherein  
the cytokine is Interleukin-2 and Interleukin-12.

-29-

7. A sustained-release device as claimed in claim 5 wherein the implant contains an antioxidant for suppressing the conversion of macrophages into foam cells, the implant being constructed and arranged to deliver the antioxidant for at least two months.

8. A sustained-release device as claimed in any one of claims 1-7 wherein the antigen is associated with a pathogen selected from the group consisting of:

human immunodeficiency virus, cytomegalovirus, Epstein Barr virus, Hepatitis virus, Herpes virus, Influenza, Human papilloma virus, mycobacteria, Pneumocystis carinii, Cryptococcus neoformans, Candida and mycoplasma.

9. A sustained-release device as claimed in claims 1, 2, 3, 4, 5, 6 or 7 wherein the antigen is associated with human immunodeficiency virus.

10. A sustained-release device as claimed in claims 1, 2, 3, 4, 5, 6 or 7 wherein the implant includes a melted and recrystallized carrier and the antigen is captured in a crystalline matrix of the melted and recrystallized carrier.

11. A sustained-release device as claimed in claims 1, 2, 3, 4, 5, 6 or 7 wherein the antigen is a peptide.

12. A method for stimulating cell-mediated immunity in a subject comprising:

inducing by implanting a foreign body in the subject the formation of a subcutaneous fibrous capsule defining a chronic, local inflammatory site, and

presenting continuously for at least two months to said site an antigen associated with a pathogen at a dose appropriate for stimulating cell-mediated immunity but at a dose that does not induce tolerance.

-30-

13. A method as claimed in claim 12 further comprising delivering continuously for at least two months to said site an agent for suppressing the conversion of macrophages to foam cells.
14. A method as claimed in claim 13 further comprising delivering continuously for at least two months to said site an antioxidant for suppressing the conversion of macrophages to foam cells.
15. A method as claimed in claim 12 further comprising delivering continuously for at least two months to said site a cytokine for stimulating cell-mediated immunity.
16. A method as claimed in claim 15, further comprising delivering continuously for at least two months to said site an agent for suppressing the conversion of macrophages to foam cells.
17. A method as claimed in claim 12, 13, 14, 15, or 16 wherein the antigen is associated with a pathogen selected from the group consisting of:  
human immunodeficiency virus, cytomegalovirus, Epstein Barr virus, Hepatitis virus, Herpes virus, Influenza, Human pampalloma virus, mycobacteria, Pneumocystis carinii, Cryptococcus neoformans, Candida and mycoplasma.
18. A method as claimed in claim 12, 13, 14, 15, or 16 wherein the antigen is associated with human immunodeficiency virus.
19. A method as claimed in claim 12, 13, 14, 15, or 16 further comprising removing cells from the subject to identify a subject-specific immuno-dominant peptide associated with said pathogen and wherein said antigen is said subject-specific immuno-dominant peptide.



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 94/06394A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 A61K9/00 A61K9/16

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 11030 (SCHERING CORPORATION) 9 July 1992	1,2,5,8, 11-13, 15,17 6,10
Y	see claims 1-4,8 see page 1, line 24 - page 2, line 2 see page 5, line 24 - page 6, line 4 see page 8, line 5 - line 12 ---	
Y	WO,A,92 20325 (ENDOCON INC.) 26 November 1992 see claims 1-4,11,20 see page 17, line 22 - page 19, line 1 -----	6,10

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

6 October 1994

Date of mailing of the international search report

18. 10. 94.

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Ventura Amat, A

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/ 06394

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
**Remark: Although claims 12-19 are directed to a method of treatment of the human body the search has been carried out and based on the alleged effects of the composition.**
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US 94/06394

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9211030	09-07-92	AU-A- 9161591	22-07-92
		CN-A- 1063227	05-08-92
		CZ-A- 9301199	16-02-94
		EP-A- 0563254	06-10-93
		JP-T- 6503830	28-04-94
-----			
WO-A-9220325	26-11-92	AU-A- 1979392	30-12-92
		EP-A- 0584220	02-03-94
-----			